CLAIMS

We claim:

1. A method for reconstituting IKK in yeast comprising the steps of:
   a. subcloning IKK subunit genes into yeast expression vectors;
   b. transforming said yeast expression vectors into yeast;
   c. growing said yeast in a selective liquid media; and
   d. controllably inducing the expression of said IKK subunits by means of inducible promoters.
2. The method of claim 1, further comprising the steps of:
   a. lysing said yeast;
   b. extracting said IKK protein; and
   c. purifying said IKK protein.
3. The method of claim 1, wherein said yeast expression vectors contain a selection marker.
4. The method of claim 2, wherein said selection marker is leucine, histidine, tryptophan, or uracil.
5. The method of claim 1, wherein said yeast expression vectors contain a tag.
6. The method of claim 1.a, wherein said tag is myc, HA, or FLAG 6his.
7. The method of claim 1, wherein said yeast expression vectors contain an inducible promoter or a constitutive promoter.
8. The method of claim 1.a, wherein said inducible promoter is methionine or galactose.
9. The method of claim 1.a, wherein said constitutive promoter is alcohol dehydrogenase.
10. The method of claim 1, wherein said IKK subunit is IKKα.
11. The method of claim 1, wherein said IKK subunit is IKKβ.
12. The method of claim 1, wherein said IKK subunit is IKKγ.
13. The method of claim 1, wherein said IKK subunits are a combination of IKKα, IKKβ, and IKKγ.

14. The method of claim 1.a, 1.a or 1.a wherein said IKKα and IKKβ subunits are subcloned into pESC ura or pESC trp vectors wherein a galactose promoter region is replaced with a met promoter from a leu(met) vector.

15. The method of claim 1.a or 1.a, wherein said IKKγ subunit is subcloned into said leu(met) vector.

16. The method of claim 1.a or 1.a, wherein said IKKγ subunit is subcloned into the pES 86(+) expression vector wherein constitutive expression is induced under the alcohol dehydrogenase promoter.

17. The method of claim 1, wherein said yeast is *Saccharomyces cerevisiae.*

18. The method of claim 1, wherein said IKK is mammalian IKK.

19. The method of claim 1.a, wherein said mammalian IKK is human IKK.

20. The method of claim 1, wherein said vectors are plasmids, small yeast chromosomes or cosmids.

21. The method of claim 1, wherein said selective liquid media is an non-inducing drop-out media.

22. The method of claim 1, wherein said purified IKK protein is substantially homologous to IKK isolated from wild-type cells.

23. The method of claim 1, wherein said purified IKK protein is mutated.

24. A heterologously expressed IKK complex, wherein said IKK is expressed by yeast.

25. The composition of claim 24, wherein said IKK complex is comprised of IKKα, IKKβ, and IKKγ subunits.

26. The composition of claim 24, wherein said IKK complex is produced by the method of claim 1.

27. A heterologously expressed IKK complex, wherein said IKKγ protein subunit regulates phosphorylation of serine residues in the activation of T loop kinase domain of IKK catalytic subunits.

28. The method of claim 27, wherein said IKK complex is activated by the dephosphorylation of γBD serines.

29. A yeast cell containing an expressible copy of a gene encoding a subunit of IKK.

30. The yeast cell of claim 1.a which is transformed with a yeast expression vector which contains the expressible copy of the gene encoding IKKα, IKKβ, or IKKγ.

31. The yeast cell of claim 1.a which is transformed by the method of claim 1.

32. A method for identifying upstream regulators of IKK complex, comprising the steps of:

a. mutating the genes of one or more said IKK subunits;

b. subcloning genes for IKK subunits into yeast expression vectors;

c. transforming said yeast expression vectors into yeast;

d. growing said yeast in a selective liquid media;

e. controllably inducing the expression of said IKK subunits by means of inducible promoters;

f. lysing said yeast;

g. extracting said IKK protein;

h. purifying said IKK protein; and

i. comparing kinase activity of said IKK protein with wild type IKK.

33. The method of claim 32, wherein said mutation is on a binding domain.

34. The method of claim 1.a, wherein said mutation mimics the biochemical characteristics of said binding site when bound.

35. The method of claim 1.a, wherein said mutation prevents binding at said domain site.

36. The method of claim 32, wherein said mutation changes serines to alanines.

37. The method of claim 32, wherein said mutation changes serines to glutamic acid.

38. A method for assaying IKK activity in situ in yeast comprising the steps of:

a. subcloning genes for IKK subunits into first yeast expression vectors;

b. transforming said first yeast expression vectors into yeast;

c. subcloning HeLa cell cDNA into second yeast expression vectors;

d. transforming said second yeast expression vectors into said yeast;

e. replica plating said yeast;

f. growing said yeast on membranes on selective non-inducing medium

g. inducing said yeast to produce IKK protein;

h. fixing said IKK protein;

i. probing said IKK protein with IKKβ, IκBα, and Phospho- IκBα (ser 32); and

j. isolate on said membranes clones positive for IKKβ and IκBα and negative for Phospho- IκBα (ser 32).

39. The method of claim 1.a, further comprising the step of sequencing said positive clones.

40. The method of claim 1.a, further comprising the steps of:

a. transforming said positive clone into yeast;

b. growing said yeast in a selective liquid media;

c. controllably inducing the expression of said clones by means of inducible promoters.